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A STUDY ON THE INHIBITORY EFFECT OF MATRINE ON GASTRIC CANCER SGC-7901 CELLS

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Abstract

The objective of this paper was to investigate the antitumour mechanism of action of matrine by studying its inhibitory effect on gastric cancer SGC-7901 cells. SGC-7901 cells were chosen, and cell-killing capacity of matrine on gastric cancer SGC-7901 cells was determined using MTT assay and single PI staining assay. The results showed that matrine had an inhibitory effect on gastric cancer SGC-7901 cells, which was somewhat dose-dependent. The study concluded that matrine has a significant in-vitro inhibitory effect on SGC-7901 tumour cells, influences cell cycle of SGC-7901 cells, and induces their apoptosis.

Key words: Matrine; SGC-7901; MTT assay

Introduction

Matrine is an alkaloid extracted and separated from leguminous plants *Sophora flavescens* Ait., *S. alopecuroides* L. or *S. subprostrata* Chun et T. Chen. As a traditional Chinese medicine, *Sophora flavescens* Ait. has anti-bacterial and anti-cancer effects and is widely used in clinical settings (Li et al., 2002). The main effective constituents in *Sophora flavescens* Ait. are alkaloids (Cui et al., 2007), of which the representative constituent is matrine. Modern experimental studies have demonstrated that matrine has anti-tumour and immunomodulatory effects (Gao & Deng, 2010). This paper aims to explore the inhibitory effect of matrine on SGC-7901 tumour cells in mice and its mechanism of action through experimental study and by modern pharmacological methods.

Materials and Methods

Reagents

The materials and reagents used for the experiment included KM mice with body weight (20 ± 2) g, half male and half female, purchased from the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences; matrine injection (manufacturer: Yuhuang Pharmaceutical Co., Ltd., Liaoning, batch number: 20101206).

Cell cultivation

Gastric cancer SGC-7901 cells were placed in RPMI 21640 complete culture medium and cultured in an incubator set at 37°C, 5% CO₂ with saturated humidity. Cells in the logarithmic growth phase were collected and used in the experiment.

Detection of cell proliferation inhibition rate by MTT (Zhang et al., 2002)

100 μ L of logarithmic growth phase gastric cancer cells (2×10^3) were taken and added to 96-well plates. After cultivation for 48 h, different concentrations of matrine (30, 60, 120 mg/L) were added, where DMSO in each concentration group was 2 % (v/v). The control group was only added with the corresponding volume of culture medium containing 2 % (v/v) DMSO. Four replicate wells were set up for each group, and the plates were cultured in the incubator set at 37°C, 5% CO₂ with saturated humidity. 24 h, 48 h and 72 h were taken as the detection time points, and MTT assay was performed according to kit instructions. 4 h before the termination of the experiment, each well was added with 10 μ L of MTT. At the end of the cultivation, supernatant was discarded. 150 μ L of DMSO was added, plates were shaken well with a shaker, and OD value of each well was measured at wavelengths of 578 nm and 690 nm using an ELISA reader. Tumour cell inhibition rate was calculated as follows:

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Tumour cell inhibition rate (%) = (average OD value of the control group - average OD value of the experimental group) / average OD value of the control group \times 100%.

Detection of cell cycle and apoptosis

Detection of cell cycle and apoptosis rate of SGC-7901 cells by single PI staining: 100 μ L of logarithmic growth phase gastric cancer cells (2×10^3) were taken and added to 96-well plates. After cultivation for 48 h, different concentrations of matrine (30, 60, 120 mg/L) were added, and the cultivation was continued for another 48 h. The gastric cancer cells which were effected by different concentrations of matrine were then collected, and the cell concentration was adjusted to 1×10^6 cells/100 μ L. The cells were washed twice with PBS and resuspended with 250 μ L of binding buffer. 195 μ L of cell suspension was taken and added with 5 μ L of AnnexinV/FITC. They were then added with 5 μ L of 20 μ g/mL PI dye and stained at 4°C for 15 min in dark conditions, which was then analysed by flow cytometry.

Statistical methods

Anova and t-test on the data of cytological experimental results were performed using SPSS 17.0 statistical software. $P < 0.05$ indicates the difference was statistically significant.

Results

Inhibitory effect of matrine on proliferation of SGC-7901 cells

The experiment showed that different concentrations of matrine samples had significant inhibitory effects on proliferation of gastric cancer cells, and were time- and dose-dependent, see Table 1.

Table 1: Inhibitory effect of matrine on proliferation of SGC-7901 cells (%)

	Time-dependence (120 mg/L) (h)			Dose-dependence (mg/L) (72 h)		
	24	48	72	30	60	120
Inhibition rate	12 \pm 1.3	24 \pm 2.4*	59 \pm 1.6*	29 \pm 1.6	40 \pm 1.3**	59 \pm 1.5**

Comparison with 24 h; * $P < 0.05$; comparison with 30 mg/L, ** $P < 0.05$

Effect of matrine on apoptosis rate of gastric cancer cells

As can be seen from Table 2, after 48 h of intervention with 30, 60 and 120 mg/L matrine, apoptosis proportion of SGC-7901 cells rose and apoptosis rate gradually increased. Compared with the control group, the results for three sample groups were all significantly different, and were statistically significant.

Table 2: Detection of apoptosis rate after 48 h action of matrine by Annexin V/PI assay (%)

Time (h)	Control group	30 mg/L	60 mg/L	120 mg/L
48	3.54 \pm 0.47	12.89 \pm 0.57*	22.34 \pm 0.76**	34.59 \pm 0.51**

Comparison with the control group, * $P < 0.05$, ** $P < 0.01$

Effect of matrine on cell cycle of gastric cancer cells

After action of matrine, a number of S-phase SGC-7901 cells were significantly reduced compared with the control group, and number of G₂/M phase cells increased significantly, demonstrating that matrine can arrest cells in G₂ phase, reducing the transition of cells to M phase (see Table 3).

Table 3: Effect of matrine on cell cycle distribution of SGC-7901 cells

Group	G ₀ /G ₁	S phase	G ₂ /M
Control group	36.9	23.8	40.2
30 mg/L	26.4	12.7	66.9
60 mg/L	22.3	9.4	72.8
120 mg/L	20.6	8.2	75.6

Discussion

Compared with the world average level, the incidence of gastric cancer has been high in China. Gastric cancer mortality ranks third among all cancers, second only to lung cancer and liver cancer (Yang et al., 2005). The reasons for this are various, but mainly because the early gastric cancer mostly has no symptoms or only has mild symptoms. When clinical symptoms are apparent, the lesion has progressed to the advanced stage (Jorgen et al., 1988). Gastric cancer mortality is 25.2 / 0.1 million (male: 32.8 / 0.1 million, female: 17.0 / 0.1 million) in China, accounting for 23.2% of all cancer deaths, and ranks first among cancer deaths (Li et al., 1997). The treatment of gastric cancer includes surgical resection and comprehensive chemoradiotherapy diagnosis and treatment (Dirk et al., 1997), but the survival rate has not been significantly improved, with a 5-year survival rate of only 10% - 15%.

Sophora flavescens Ait. is a common medicine in China, usually used as a traditional Chinese medicine for the treatment of pharyngeal, dermatological and gynaecological diseases. *Sophora flavescens* Ait. has been valued by doctors over the ages with its good efficacy (DAI et al., 2011). Modern medical studies have shown that matrine has an anti-tumour activity both in vitro and in vivo, and that it can effectively inhibit tumour cell proliferation and metastasis, while promoting tumour apoptosis, inducing differentiation, and improving the immunity of patients (Wang and Long, 2005; Kamaliddinov et al., 1969).

In this experiment, gastric cancer SGC7901 cells were taken as the studying object. Fast and accurate quantitative analysis of apoptosis level was carried out by flow cytometry (Vermes et al., 1995; Dolzhanskiy and Basch, 1995). The inhibitory effect of matrine on gastric cancer cells was observed, and its mechanism of action was explored from the perspectives of cell cycle and induction of apoptosis. The results suggested that different concentrations of matrine have significant inhibitory effects on gastric cancer SGC-7901 cells, which showed time- and dose-dependence.

Apoptosis refers to the autonomous orderly death of cells controlled by genes in order to maintain homeostasis; it was firstly proposed by Professor Kerr according to morphological characteristics as early as 1972 (Kerr et al., 1972). Apoptosis differs from cell death, which plays an important role in the activities of histological differentiation, organ development, homeostasis, etc. (PascalMeier et al., 2000) It is generally believed that the generation of tumour cells is due to uncontrolled growth and excessive proliferation of cells; while from the perspective of apoptosis, it is considered to be the results of inhibited apoptosis mechanism of tumours, which make normal cell death impossible (John and Fadok, 2000).

The study on tumour inhibitory mechanism of matrine centred on the effect of matrine on apoptosis and cell cycle. The results of this study showed that matrine has an obvious apoptosis inducing effect on SGC-7901 cells. After 48 h of action, the apoptosis rate significantly rose with the increase of matrine concentration, which was similar to the reported literature (Zhang et al., 2000). As for the effect on cell cycle, the results of this study suggested that matrine can arrest cells in the G₂ phase, and reduce cell transition to the M phase, which showed a concentration-dependence.

In conclusion, the results of this study showed that matrine has a time- and concentration-dependent inhibitory effect on gastric cancer SGC-7901 cell lines. This inhibitory effect is related to the arrest of cell cycle at G₂ phase and induction of apoptosis. The relevant molecular mechanism however needs further in-depth study.

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